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The Histological Structure of the Normal and the Hyperplastic Thyroid in *Rasbora lateristriata* (Bleeker).

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(Plates I-III).

In an earlier publication (Smith, Coates & Strong) it was noted that hyperplastic conditions of the thyroid among small tropical fishes of the New York Aquarium occurred only in rare instances. Among approximately 400 species there were two examples of thyroid growths in the course of a period of observation lasting five years. These conspicuously enlarged goitres occurred in *Rasbora lateristriata* (Bleeker) and in *Heterandria formosa* Agassiz. They were sporadic forms of the disease and progressive in nature. Death occurred between six and eight weeks after the thyroid tumors were first observed. In order to study in greater detail the alterations in the diseased thyroid, a complete serial section of the entire head of a specimen of *Rasbora lateristriata* with thyroid hyperplasia has since been made. In addition, complete serial sections of four normal adult fishes of the same species, two male and two female, were prepared for purpose of comparison. The histologic observations on the normal and the hyperplastic thyroids are recorded and illustrated in the present paper.

A large part of the thyroid studies in fishes has been done on the Salmonidae, as these fishes are subject at times to a diseased enlargement of this gland, usually in the form of a benign hyperplasia, yet occasionally showing morphologic changes suggesting malignancy. A number of years ago, Gudernatsch (1911) described the normal structure of the thyroid gland in twenty-nine species of teleosts. By a systematic comparison of all these species this author found that there exists a wide variation in the thyroid gland of fishes, with a continuous series of transitions from one form to another. The thyroid gland of fishes, often invisible to the eye, is usually detected by microscopic examination. The actual position of the thyroid gland is described by Gudernatsch as lying below the floor of the pharynx, in the body of the tongue, between the gill arches, extending posteriorly near the origin of the third and fourth branchial arteries as they arise from the ventral aorta. The paired muscles of the sternohyoideus extend below the gland, while above the gland lie the bony and soft parts of the floor of the pharynx. The thyroid, therefore, lies in a relatively closed compartment whose separate structures are subject to con-

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siderable variation. It is not surprising that the thyroid tissue itself was found to possess differences in size and distribution in individuals of the same species. For example, Gudernatsch found that in twelve weak-fish (*Cynoscion*), all differed in the extent and position of their respective thyroids.

It is well known that the thyroid gland of fishes cannot be regarded as a solid compact encapsulated organ as in higher vertebrates, but rather as a collection or grouping of follicles. The more densely arranged follicles lie in the neighborhood of the aorta or the branchial arteries particularly where these branches arise from the aortic stem. In Gudernatsch's studies, the thyroid follicles were most abundant in the region of the second gill arteries. The roots of the last gill branches seemed to have the least number of follicles. Usually the thyroid tissue lies ventral to the aorta, yet in some fishes dense clusters of follicles are located both dorsal and ventral to the aorta. Near the periphery, follicles are more commonly scattered in their arrangement.

It is of considerable anatomic interest that thyroid tissue in fishes follows a distribution along the main blood channels running between the heart and the respiratory tissue of the gills. It is believed that in the fish embryo, thyroid tissue lying originally between the first and the second gill branches later in development migrates in caudal, cephalic, and lateral directions to assume various other new yet permanent positions along the region of the aorta. This embryological migratory tendency along blood vessels exhibited by thyroid tissue may account for certain displaced thyroid follicles being found later in life in the domain of the more distant blood vessels of the gills. Such a distribution is not at all uncommon in the case of the trout under apparently normal conditions. The finding of normal thyroid tissue in the gills has offered considerable difficulty in interpreting the meaning of the larger hyperplastic thyroid masses involving gill structures when the thyroid gland as a whole responds to a stimulus causing a diffuse overgrowth.

At the time of Gudernatsch's paper on the normal thyroid in teleosts, a great deal of interest was manifest in the enlarged thyroid or goitre occurring in the trout. This disease made its appearance in this country and in Europe in epidemic and sporadic form. The lesions of fish goitre were extensively studied by Gaylord and Marsh (1914), Marine and Lehnhart (1910), Plehn (1902), Pick (1905), Pick and Poll (1903). The gross lesion makes its appearance as a pinkish globular swelling below the floor of the mouth, and later as a conspicuous swelling on the ventral surface in the region of the gills. Such goitres were known to regress, yet at times they showed a progressive course with a fatal issue. Various causative factors were suggested as important in their production, such as dietetic insufficiencies, lack of iodine, undetermined bacterial or virus infection from sluggish or contaminated water. As the disease in some of the fishes, as already stated, proved to be a progressive one and the goitre became a conspicuous tumor at times involving, microscopically, cartilage, bone, muscle or epithelium, some investigators attributed neoplastic features to this atypical form of growth, and the term "so-called carcinoma of the thyroid" came into descriptive use. It was pointed out that such thyroid tumors remained strictly localized. The few instances of associated isolated secondary growths described for the mandibular, gill or anal region, could be interpreted as embryologically displaced thyroid tissue in a state of hyperplasia. The evidence now points to the fact that nearly all types of fish tumors remain localized, irrespective of the histological characteristics, or the organ involved, and that secondary growths or metastases do not occur with the frequency among fishes that they do in the case of tumors in birds and mammals.

In our own investigation of the thyroid in *Rasbora lateristriata*, comprising four normal fishes, two male and two female, and the one case of thyroid hyperplasia, the entire head was fixed in 10 percent. formalin, and after decalcification, serial sections were prepared by the paraffin method. The stain used was hematoxylin and eosin.

The normal thyroid gland of this small "tropical fish" measuring about 3 cm. is invisible to the naked eye, but can be definitely located microscopically in the region anterior to the heart in close relation to the aorta. As is usual in the teleost, thyroid tissue does not form a compact gland. It consists in this fish of a very few small follicles, about 10 to 12 in number, strung along the main vascular trunk or its immediate branches. (Plate I, Figs. 1-4). The follicles are complete and do not communicate with each other. They are oval or circular in cross section and contain a small amount of eosinophilic staining colloid material, in places shrunken away from the lining epithelium as a result of the action of the fixative. The epithelium is a delicate one of a very low columnar or flattened type. The cytoplasm is clear, at times slightly vacuolated, while the nucleus of the cell is round or oval and not deeply stained. The epithelium is supported by fine fibrillar connective tissue showing here and there a narrow elongated nucleus. This fine fibrous tissue may send out processes uniting follicles to each other or to the extremely thin wall of adjacent blood vessels. No colloid droplets are visible in the epithelium or neighboring tissue spaces, blood, or lymph vessels. The larger thyroid follicles measure from 50 to 90 microns in diameter. There seem to be no clusters or nests of thyroid epithelium. No clues are suggested as to the manner of growth or regression of the thyroid follicle in this small fish. A few small blood vessels are found near thyroid tissue and an occasional small nerve trunk. No thyroid tissue was distributed in the substance of the gills. There was no essential difference between the male and the female thyroid.

Contrasting with the normal thyroid tissue, the hyperplastic thyroid growth offered a wide difference in appearance and distribution of its structural elements. The thyroid tumor itself measures 2.5 mm. in diameter, whereas the thyroid follicles in the normal fish occupy an area estimated as not much more than .1 mm. The goitre forms a somewhat irregular-shaped mass without a capsule. (Plate II, Fig. 5). The mass is composed in part of small compact thyroid follicles, in part of a diffuse continuous collection of larger follicles in various stages of distention with colloid. (Plate II, Fig. 6). The largest follicles measure as much as 550 x 190 microns. A rough estimate indicated that in the normal fish not more than 10 follicles were present in a cross section, whereas the thyroid tumor possessed perhaps as many as 3,000 follicles in a transverse section through its widest diameters. In the most compact part of the tumor, epithelial cells are very small with a small light-staining nucleus, while follicles distended with colloid are lined by very low columnar or flat cells. The irregular infolding of epithelium seen in human exophthalmic goitre is not present. Occasionally there is a papillary fold of epithelium supported by a delicate core of connective tissue which projects from the wall of a distended or cystic follicle into the colloid containing lumen. There are a few places where a point of communication exists between two adjacent cystic follicles (Plate III, Fig. 12). The rule is that where follicles are once formed, they remain globular and complete without any evidence of branching. For the most part follicles lie in contact, with a small amount of very fine connective tissue interposed. There is no evidence of fibrosis.

Whereas in the normal fish lymphoid cells are practically absent, in the hyperplastic specimen scattered collections of lymphoid cells are not uncommon. Such areas of lymphoid cells suggest the existence of an associated mild chronic inflammation. We found no inclusion bodies either nuclear or cytoplasmic.

Conspicuous in the diseased thyroid is the greatly increased vascularity. In all parts of the thyroid tumor are found small deeply engorged veins, representing a blood supply greatly increased over the normal. (Plate II. Fig. 6). There appeared to be no evidence that lymphatic vessels were correspondingly increased in number. The thyroid tumor does not show sufficient mitoses to indicate a rapid growth, nor are there changes suggesting degenerative processes or actual necrosis.

Infiltration of muscles, cartilage and bone is frequently encountered. (Plate III, Figs. 9, 10). The destructive assault upon gill tissues is particularly noteworthy (Plate II, Figs. 7, 8), as in the normal fishes no traces of thyroid tissue could be identified as occupying gill structures. In the caudal direction, the thyroid mass encroaches on the aorta near the heart (Plate III, Fig. 11). Here as in the gills, mechanical pressure caused by the thyroid mass might seriously impair the function of important vascular structures. It seems extremely doubtful that with such advanced replacement of gill tissues a complete regression and restoration to normal could be expected even if the necessary physiological conditions were ever re-established as, for example, by the experimental supply of sufficient iodine. Whether or no destructive invasion of normal tissues associated with the advancing thyroid growth represents in any sense a low grade of neoplasia is a matter of individual interpretation. Further studies of sporadic forms of thyroid growth in these small fishes would help to determine this point.

Summary.

The histology of the normal thyroid of *Rasbora lateristriata* is described and contrasted with an instance of massive hyperplasia of the thyroid occurring in the same species. The disease of the thyroid occurred in sporadic form. It was progressive and fatal in the course of two months. The excessive growth of the thyroid in this species of small fish destroyed and replaced gill structures, thus impairing the function of this important tissue.

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EXPLANATION OF THE PLATES.

Plate I.

Fig. 1. Section through the head of normal *Rasbora lateristriata* made at the level of the eyes. The small compartment containing thyroid tissue is indicated by the circle, *O*. M, floor of the mouth. x 15. Compare with the hyperplastic mass of thyroid seen in Fig. 5, Plate II.

Fig. 2. Four normal thyroid follicles, 1, 2, 3, 4, lying slightly dorsal to the aorta, A, near the origin of the branchial arteries, X and Y. x 125.

Fig. 3. Cluster of ten normal colloid-containing follicles dorsal to the aorta, A. x 200.

Fig. 4. Three normal thyroid follicles attached to a vein, V. A marks the aorta. x 500.

Plate II.

Fig. 5. Low power magnification of hyperplastic thyroid mass, T. x 13. The section is taken through the head at the level of the optic nerves. Compare with the normal thyroid area seen in Fig. 1, Plate I.

Fig. 6. Hyperplastic tissue with follicles of various sizes, with blood vessels at V and V′. x 175.

Fig. 7. Hyperplastic thyroid mass replacing gill tissue, G and G′. x 45.

Fig. 8. Thyroid follicles occupying gill structures. x 125.

Plate III.

Fig. 9. Hyperplastic thyroid invading muscle fibres, M. x 250.

Fig. 10. Hyperplastic thyroid infiltrating bony structures, B. x 125.

Fig. 11. Thyroid mass encroaching upon the aorta, A, near the region of the heart. x 58.

Fig. 12. Hyperplastic follicles with vacuoles in the colloid. Colloid material in follicles A and B appeared to be continuous through a break in the septum, S. x 250.